

- (c) contacting cells of said epithelial tissue with said packaged viral vector under conditions permitting the uptake of said packaged viral vector by said cells and expression of said polypeptide therein;

whereby increased permeability of said epithelial tissue facilitates improved viral transduction of said cells, which in turn facilitates expression of said polypeptide.

69. (Amended) The composition of claim 68, further comprising a packaged viral vector.
70. (Amended) A method for transforming epithelial cells with a viral vector comprising delivering to said epithelial cells a packaged viral vector and EGTA in a hypotonic solution.

REMARKS

I. Status of the Claims

Claims 1-70 are pending in the application. Claims 13-25, 57-59, 61 and 62 are withdrawn from consideration. Thus, claims 1-12, 26-56, 60 and 63-70 are under examination and stand rejected. The specific grounds for rejection, and applicants' response thereto, are set out in detail below.

II. Rejection Under 35 U.S.C. §112, First Paragraph

Claims 1-12, 26-56, 60 and 63-70 are rejected under the first paragraph of §112 as lacking an enabling disclosure. The examiner has provided what can only be termed an

extensive discussion of this topic, and applicants appreciate the examiner's thorough analysis of the subject matter of the application. However, respectfully, applicants submit that the examiner's position is incorrect, both factually and legally, as explained in depth in the following pages.

At the outset, it is important to understand that several issues central to the pathogenesis and treatment of cystic fibrosis remain unresolved scientifically in the time since the CFTR gene was discovered in 1989. The disease pathogenesis remains controversial, as do approaches for new therapies, including gene transfer. However, applicants believe the examiner has provided a slanted view of the prior art, focusing on the most negative aspects of gene therapy, and ignoring more favorable reviews. In addition, the action stands as an indictment of gene therapy generally, which applicants believe is not representative of the view of those of skill in the art, *e.g.*, Crystal (1999) (C100).

The present methods represent a significant advance in the application of viruses to gene transfer involving epithelia. In particular, the present specification contains irrefutable evidence, both *in vitro* and *in vivo*, that indicates the claimed invention provides a dramatic improvement in viral gene transfer into epithelial cells. Thus, in many settings including cystic fibrosis, the effectiveness of gene transfer can be increased using this invention.

A. *"The prior art teaches that it is not known which cells must be transfected with CFTR expression vectors in order to treat CF."*

It is generally true that it remains controversial which specific cell types must be transduced in order to correct the CF defect. However, there are significant data supporting the idea that correction of chloride transport defect is fundamental. Loss of cyclic AMP activated chloride conductance in airway epithelia is the fundamental physiologic hallmark of cystic

fibrosis. The CFTR chloride channel has been studied extensively, and there is general consensus that one key function of this channel is to transport chloride. This function is defective in cystic fibrosis. From a prevailing perspective, it may not be necessary to know which cell types to transduce as long as the end result is restoration of chloride transport. This is the general strategy that is being taken by the field. What is required is restoration of CFTR function (chloride conductance), which applicants have achieved (below).

The present inventors *in vitro* data using primary cultures of airway epithelia derived from patients with cystic fibrosis clearly show that it is possible to correct the chloride transport defect in this model for 11 months' duration with retroviral vectors (see Figure A, attached). Based on the turnover of epithelial cells in this model, in which cells are slowly proliferating, these data suggest that the results stem from transduction of a population of cells with progenitor capacity that have gradually repopulated the epithelium. This duration of expression is unprecedented in the cystic fibrosis gene literature and speaks to the efficacy of the present invention and the ability of retroviral transgenes to integrate and persist. These results also verify that a retroviral vector integrated into the host cell genome can persist in a population of progenitor cells in a manner sufficient to correct the chloride transport defect. Given this ability, applicants submit that the instant specification is sufficiently enabling, even with the caveat discussed above regarding target cells.

The examiner focuses on the potential of correcting the CFTR defect in submucosal gland epithelia. It is true that submucosal gland cells express CFTR in greater abundance than surface epithelia. This has led some investigators to speculate that the role of CFTR in the submucosal gland cells may be critical in disease pathogenesis. However, there is no universal

agreement in this regard and the role of submucosal glands in disease pathogenesis continues to be debated.

The examiner also argues that it is critically important that a vector be delivered by the systemic circulation in order to target the submucosal glands rather than deliver a vector topically through the airway lumen. Others have tried to deliver viral vectors systemically to target airway and submucosal gland epithelia, and the efficiency of this approach has been low. Lemarchand *et al.* (1994) (C102). Furthermore, there is evidence from studies in transgenic animals to suggest that precise delivery of the therapeutic gene to its normal cell type of expression may be required for therapeutic benefit in CF. For example, Zhou *et al.* (1994) (C105) showed that expressing CFTR in the intestinal surface epithelia using the FABP promoter corrected the fatal intestinal phenotype in CFTR-null mice despite the fact that the CFTR expression was directed to surface epithelia rather than the crypt epithelia in which it is normally expressed in high abundance. Applicants submit that this is not only the best evidence of cell-type non-specificity, it is the *only* evidence of record on this point. Thus, speculation of certain commentators notwithstanding, applicants believe that this study argues against the examiner's position.

B. "It is also unclear how many cells must be transfected and what level of gene expression is required to achieve therapy."

While it is true that gene therapy for CF is not being routinely practiced at this time, and that there is no detailed protocol to which applicants can point, such a situation does not preclude enablement. There are *in vitro* studies showing that adding between 6% and 10% normal cells to a population of CF cells corrects the chloride transport defect. Johnson *et al.*, 1992 (C101). Thus, it is not the case that correction of the CFTR defect in 100% of cells would be required,

although the precise number of cells required *in vivo* is not known. However, though such potential knowledge gaps may exist, this does not preclude a finding that the current invention is enabled. "We hold as we do because it is our firm conviction that one who has taught the public that a compound exhibits some desirable pharmaceutical property in a standard experimental animal has made a significant and useful contribution to the art, even though it may eventually appear that the compound is without value in the treatment of humans." *In re Krimmel*, 130 USPQ 215 (CCPA 1961).

C. *"Boucher (1999) teaches that it is likely that the percentage of epithelial cells requiring functional correction to restore normal chloride ion transfer in vivo may well exceed 10%"*

The examiner has placed considerable weight on the study of Boucher, which reported that more than 10% correction of CFTR defect may be required to accomplish a therapeutic goal. This hypothesis is based largely on work at a single center, and is not accepted as the fundamental issue facing CF therapy. These studies used adenoviral vectors in a time before the receptor for the virus was recognized and, not surprisingly, the transfer efficiency was low. The present invention directly affects this problem, but increasing the viral transduction of target cells. In the inventors' own publications, *in vivo* studies have achieved up to 14% transduction of tracheal epithelia, and up to 10% of epithelia in small airways. Wang *et al.* (1999) (C103); Wang *et al.* (2000) (C104). In an unpublished manuscript, transduction of rabbit tracheal epithelia exceeds 10% in some areas. Taken together with the showing that nasal perfusion of respiratory epithelia with an EGTA solution decreased transepithelial electrical potential, indicating opening of tight junctions, these papers indicate a tremendous potential for gene therapy in humans.

5

D. "[T]he accumulation of mucus associated with the CF in humans ... impedes vector access to epithelium"

It is argued that mucus in patient airways presents yet another hurdle to the use of gene therapy. However, if the therapy is applied to infants and young children prior to the development of this symptom, the airways will not present the same barriers. As such, this fact cannot preclude a finding of enablement for the simple reason that it does not apply to patients across the board.

III. Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 1-12, 32, 33, 48-52 and 68-70 are rejected under the second paragraph of §112 as being indefinite.

Claims 1-12 and 70 are said to be indefinite as the claims allegedly fails to present a step correlating with the preamble – increasing susceptibility to viral infection. Applicants traverse, but in the interest of advancing the prosecution, the claims have been amended. Claim 1 now includes a “whereby” clause, and the preamble of claim 70 has been modified.

Claim 8 is said to indefinite for failing to further limit claim 7. Applicants disagree. The term “proliferative factor” is generic, and encompasses the term “growth factor,” which as an art accepted definition (see attached). For example, an oncogene could be a proliferative factor, as could a molecule such as Bcl-X_L, neither of which qualify as “growth factors.”

Claims 32 and 33 are allegedly indefinite in the recitation of the term “diseased.” Applicants traverse. The examiner argues that the “degree” of disease is not provided. Since there is not degree of disease required by the claim, applicants believe this issue is irrelevant. The tissue is either diseased or healthy, and one of skill in the art could make that determination

without undue experimentation. The examiner also that use of "diseased" with "tissue" is indefinite, citing the specific disease states listed in claim 33. Again, applicants disagree. The term "diseased tissue" is commonly used by clinicians, and these clinicians would be very well aware of which tissues would be "diseased" in each of the disease states listed in claim 33. Thus, applicants submit that the claims are sufficiently clear to apprise those of skill in the art of the metes and bounds.

Claims 68-70 are said to be indefinite for the use of the term "hypotonic." Applicants disagree, as the term is used commonly in the field. In addition, applicants have provided a definition in the specification, at page 44, lines 6-9, where it is stated that "Hypotonic solutions are defined relative to normal osmolality, or normotonic solutions. Normotonic solutions are around 280-300 mosm/kg. The hypotonic solutions according to the present invention are less than about 280 mosm/kg. One particular buffer is about 105 mosm/kg, while others are about 25-50 mosm/kg."

Claims 48 and 49 are said to lack a step concordant with the preamble. An appropriate whereby clause has been added to claim 48.

Claims 50-52 are said to lack a step concordant with the preamble. An appropriate whereby clause has been added to claim 50.

Claim 69 is said to be indefinite in use of the term "package viral vector." The suggested change has been made.

Reconsideration and withdrawal of each of the preceding rejection is respectfully requested.

IV. Rejections Under 35 U.S.C. §102(b)

A. *Halbert et al.*

Claims 1, 2, 4, 6-8, 26-31 and 48-52 stand rejected under §102(b) as anticipated by Halbert *et al.* According to the examiner, Halbert teaches increasing permeability of tracheal tissue by wounding. Applicants have clarified the present claims to indicate that the method by which permeability is increased is by application of composition that comprises a tissue permeabilizing agent. This clearly distinguishes the methods of Halbert. Reconsideration and withdrawal of the rejection is respectfully requested.

B. *Mallea et al. or Yamaguchi et al.*

Claims 38 and 43 stand rejected under §102(b) as anticipated by Mallea *et al.* or Yamaguchi *et al.* Applicants traverse the rejection but, in the interest of advancing the prosecution, the limitation of dependent claims *not* rejected over Mallea or Yamaguchi have been incorporated into claims 38 and 43. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

C. *Wunderlich et al.*

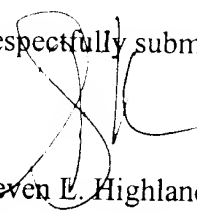
Claims 68-70 stand rejected under §102(b) as anticipated by Wunderlich *et al.* Applicants traverse. The rejection is based on the examiner's mistaken belief that the term "hypotonic solution" is not defined. As discussed above, the specification provides a clear definition of the term "hypotonic." Thus, it is incumbent upon the examiner to establish that the cited reference meets the limitation of this claim. Applicants submit that the present record is

insufficient in this regard and, therefore, that the rejection is improper. Reconsideration and withdrawal of the rejection is respectfully requested.

V. Conclusion

In light of the foregoing, applicants respectfully submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. The examiner is invited to contact the undersigned attorney at (512) 536-3184 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



Steven L. Highlander
Reg. No. 37,642
Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-3184

Date: March 7, 2002

APPENDIX A: MARKED UP COPY OF CLAIMS

1. (Amended) A method for increasing the susceptibility of epithelial cells to viral infection comprising [increasing the transepithelial permeability of epithelial tissue comprising said cells] treating said cells with composition that comprises a tissue permeabilizing agent, whereby an increase in transepithelial permeability increases the susceptibility of said epithelial cells to viral infection.
9. (Canceled) The method of claim 1, wherein increasing the intraepithelial permeability of said epithelial tissue is achieved by contacting cells of said epithelial tissue with a tissue permeabilizing agent.
38. (Amended) A composition suitable for aerosol application comprising a tissue permeabilizing agent, [and] a cell proliferative factor and a packaged viral vector.
40. (Canceled) The composition of claim 38, further comprising a packaged viral vector.
41. (Amended) The composition of claim [40] 38, wherein said packaged viral vector comprises a non-viral gene.
42. (Amended) The composition of claim [40] 38, wherein said packaged viral vector is a retroviral vector.
43. (Amended) A composition suitable for topical application comprising a tissue permeabilizing agent, [and] a cell proliferative factor and a packaged viral vector.
45. (Canceled) The composition of claim 43, further comprising a packaged viral vector.
46. (Amended) The composition of claim [45] 43, wherein said packaged viral vector comprises a non-viral gene.

47. (Amended) The composition of claim [45] 43, wherein said packaged viral vector is a retroviral vector.
48. (Amended) A method for redistributing viral receptors on epithelial cells of an epithelial tissue comprising increasing the transepithelial permeability of said epithelial tissue, whereby increased transepithelial permeability facilitates redistribution of said viral receptors on said epithelial cells.
50. (Amended) A method for expressing a polypeptide in cells of an epithelial tissue comprising:
- (a) providing a packaged viral vector comprising a polynucleotide encoding said polypeptide;
 - (b) increasing the permeability of said epithelial tissue; and
 - (c) contacting cells of said epithelial tissue with said packaged viral vector under conditions permitting the uptake of said packaged viral vector by said cells and expression of said polypeptide therein;
- whereby increased permeability of said epithelial tissue facilitates improved viral transduction of said cells, which in turn facilitates expression of said polypeptide.
69. (Amended) The composition of claim 68, further comprising a [package] packaged viral vector.
70. (Amended) A method for [increasing the susceptibility of] transforming epithelial cells [to] with a viral [infection] vector comprising delivering to said epithelial cells a packaged viral vector and EGTA in a hypotonic solution.

APPENDIX B: CLEAN COPY OF CLAIMS (UNOFFICIAL)

1. A method for increasing the susceptibility of epithelial cells to viral infection comprising treating said cells with composition that comprises a tissue permeabilizing agent, whereby an increase in transepithelial permeability increases the susceptibility of said epithelial cells to viral infection.
2. The method of claim 1, wherein said epithelial tissue is airway epithelial tissue.
3. The method of claim 2, wherein said airway epithelial tissue is bronchial tissue.
4. The method of claim 2, wherein said airway epithelial tissue is tracheal tissue.
5. The method of claim 2, wherein said airway epithelial tissue is alveolar tissue.
6. The method of claim 1, further comprising increasing the proliferation of said epithelial cells.
7. The method of claim 6, wherein increasing the proliferation of said epithelial cells is achieved by contacting said cells with a proliferative factor.
8. The method of claim 7, wherein said proliferative factor is a growth factor.
10. The method of claim 9, wherein said tissue permeabilizing agent is a hypotonic solution.
11. The method of claim 9, wherein said tissue permeabilizing agent is ion chelator.
12. The method of claim 11, wherein said ion chelator is EGTA, BAPTA or EDTA.
13. The method of claim 9, wherein said tissue permeabilizing agent is a cationic peptide.

14. The method of claim 13, wherein said cationic peptide is poly-L-lysine.
15. The method of claim 9, wherein said tissue permeabilizing agent is an occludin peptide.
16. The method of claim 9, wherein said tissue permeabilizing agent is a cytoskeletal disruption agent.
17. The method of claim 16, wherein said cytoskeletal disruption agent is cytochalasin B or colchicine.
18. The method of claim 9, wherein said tissue permeabilizing agent is ether or glycerol.
19. The method of claim 9, wherein said tissue permeabilizing agent is a neurotransmitter.
20. The method of claim 19, wherein said neurotransmitter is capsianoside.
21. The method of claim 9, wherein said tissue permeabilizing agent is FCCP.
22. The method of claim 9, wherein said tissue permeabilizing agent is an oxidant.
23. The method of claim 22, wherein said oxidant is hydrogen peroxide or ozone.
24. The method of claim 9, wherein said tissue permeabilizing agent is a mediator of inflammation.
25. The method of claim 24, wherein said mediator of inflammation is $\text{TNF}\alpha$.
26. The method of claim 1, further comprising infecting said epithelial tissue with a virus vector selected from the group consisting of a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus, a parvovirus, a papovavirus, paramyxovirus and a vaccinia virus.

27. The method of claim 26, wherein the virus vector comprises a non-viral gene under the control of a promoter active in eukaryotic cells.
28. The method of claim 27, wherein said non-viral gene is a human gene.
29. The method of claim 28, wherein said gene encodes a polypeptide selected from the group consisting of a tumor suppressor, a cytokine, an enzyme, a toxin, a growth factor, a membrane channel, an inducer of apoptosis, a transcription factor, a hormone and a single chain antibody.
30. The method of claim 26, wherein the virus vector is a replication-defective virus.
31. The method of claim 30, wherein the virus vector is a retroviral vector.
32. The method of claim 1, wherein said epithelial tissue is diseased.
33. The method of claim 32, wherein said disease is lung cancer, tracheal cancer, asthma, surfactant protein B deficiency, alpha-1-antitrypsin deficiency or cystic fibrosis.
34. The method of claim 7, wherein said proliferative factor is delivered as an aerosol.
35. The method of claim 7, wherein said proliferative factor is delivered as a topical solution.
36. The method of claim 9, wherein said tissue permeabilizing agent is delivered as an aerosol.
37. The method of claim 9, wherein said tissue permeabilizing agent is delivered as a topical solution.
38. A composition suitable for aerosol application comprising a tissue permeabilizing agent, a cell proliferative factor and a packaged viral vector.

39. The composition of claim 38, wherein said tissue permeabilizing agent is a hypotonic solution, a cytokine, a cationic peptide, a cytoskeletal disruptor, a mediator of inflammation, an oxidant, a neurotransmitter or an ion chelator.
41. The composition of claim 38, wherein said packaged viral vector comprises a non-viral gene.
42. The composition of claim 38, wherein said packaged viral vector is a retroviral vector.
43. A composition suitable for topical application comprising a tissue permeabilizing agent, a cell proliferative factor and a packaged viral vector.
44. The composition of claim 43, wherein said tissue permeabilizing agent is a hypotonic solution, a cytokine, a cationic peptide, a cytoskeletal disruptor, a mediator of inflammation, an oxidant, a neurotransmitter or an ion chelator.
46. The composition of claim 43, wherein said packaged viral vector comprises a non-viral gene.
47. The composition of claim 43, wherein said packaged viral vector is a retroviral vector.
48. A method for redistributing viral receptors on epithelial cells of an epithelial tissue comprising increasing the transepithelial permeability of said epithelial tissue, whereby increased transepithelial permeability facilitates redistribution of said viral receptors on said epithelial cells.
49. The method of claim 48, wherein said receptor is a retroviral receptor.
50. A method for expressing a polypeptide in cells of an epithelial tissue comprising:

- (a) providing a packaged viral vector comprising a polynucleotide encoding said polypeptide;
- (b) increasing the permeability of said epithelial tissue; and
- (c) contacting cells of said epithelial tissue with said packaged viral vector under conditions permitting the uptake of said packaged viral vector by said cells and expression of said polypeptide therein;

whereby increased permeability of said epithelial tissue facilitates improved viral transduction of said cells, which in turn facilitates expression of said polypeptide.

51. The method of claim 50, further comprising increasing the proliferation of cells of said epithelial tissue.

52. The method of claim 50, wherein said viral vector is a retroviral vector.

53. A method for treating an epithelial tissue disease comprising:

- (a) providing a packaged viral vector comprising a polynucleotide encoding said therapeutic polypeptide;
- (b) increasing the permeability of the diseased epithelial tissue; and
- (c) contacting cells of said epithelial tissue with said packaged viral vector under conditions permitting the uptake of said packaged viral vector by said cells and expression of said therapeutic polypeptide therein,

whereby expression of said therapeutic polypeptide treats said disease.

54. The method of claim 53, further comprising increasing the proliferation of cells of said diseased epithelial tissue.

55. The method of claim 53, wherein the diseased epithelial tissue is airway tissue.

56. The method of claim 55, wherein said diseased airway tissue is alveolar tissue, bronchial tissue or tracheal tissue.
57. The method of claim 53, wherein said disease is a cancer.
58. The method of claim 57, wherein said cancer is lung cancer.
59. The method of claim 57, wherein said cancer is tracheal cancer.
60. The method of claim 53, wherein said disease is an inherited genetic defect.
61. The method of claim 60, wherein said inherited genetic defect is surfactant protein B deficiency.
62. The method of claim 60, wherein said inherited genetic defect is alpha-1-antitrypsin deficiency.
63. The method of claim 60, wherein said inherited genetic defect is cystic fibrosis.
64. The method of claim 53, wherein said therapeutic polypeptide is selected from the group consisting of a tumor suppressor, a cytokine, an enzyme, a toxin, a growth factor, a membrane channel, an inducer of apoptosis, a transcription factor, a hormone and a single chain antibody.
65. The method of claim 53, wherein increasing the permeability of the diseased epithelial tissue comprises contacting cells of said diseased epithelial tissue with a tissue permeabilizing agent.
66. The method of claim 54, wherein increasing the proliferation of cells of said diseased epithelial tissue comprises contacting said cells with a proliferative agent.

67. The method of claim 53, wherein said viral vector is a retroviral vector.
68. A composition comprising EGTA and in a hypotonic solution.
69. The composition of claim 68, further comprising a packaged viral vector.
70. A method for transforming epithelial cells with a viral vector comprising delivering to said epithelial cells a packaged viral vector and EGTA in a hypotonic solution.